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RENAL EXCRETION OF DIGOXIN IN SWINE AND GOATS*

By

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RASMUSSEN, FOLKE, M. NAWAZ and EVA STEINESS: *Renal excretion of digoxin in swine and goats.* Acta vet. scand. 1975, *16*, 525—536. — In experiments on swine and goats the renal excretion of digoxin was examined, and it was found that the renal clearance of non-protein-bound digoxin in swine was lower than creatinine clearance which expresses filtration clearance. Correlation analysis showed that the renal clearance of digoxin in swine was not significantly influenced by the concentration of non-protein-bound digoxin in plasma and the pH of the urine, while there was a significant positive correlation between the clearance and the urine flow rate (Table 4). On the other hand, the renal clearance of digoxin in goats was significantly influenced by the concentration of non-protein-bound digoxin in plasma and by urine pH (Table 4). From these results it is concluded that glomerular filtration and back-diffusion are involved in the renal handling of digoxin in both swine and goats. In addition active tubular secretion is also involved in the renal excretion of digoxin in goats.

digoxin; renal excretion; swine; goats.

Digoxin in humans is mostly eliminated unchanged through the kidney, and several authors have demonstrated that the renal clearance of digoxin calculated from the plasma digoxin concentration in man is equal to creatinine clearance (*Bloom & Nelp* 1966; *Doherty et al.* 1968, 1969; *Ewy et al.* 1969; *Bertler & Redfors* 1972). However, digoxin is to some extent bound to plasma proteins and therefore not available for filtration and this means that digoxin is also subject to tubular secretion as it was demonstrated in humans (*Steiness* 1974).

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The purpose of this study was to examine the renal handling of digoxin in swine and goat and compare this to findings in humans.

MATERIALS AND METHODS

Eight experiments comprising 34 experimental periods were performed on 6 healthy female swine weighing 43 to 80 kg, and 10 experiments (50 experimental periods) were conducted on 6 healthy female goats weighing 28 to 56 kg.

The swine were given one intramuscular injection of digoxin (Injectabile digoxini 0.25 mg/ml) at a dose level of 8—36 µg/kg b.wt. The renal clearance was determined after 1 hr. equilibration (*Steiness et al.* 1974). Blood and urine samples were drawn before the digoxin injection and 4 times at 20 min. intervals after the equilibration (*Gyrd-Hansen* 1968).

In experiments on goats a priming dose was given intravenously (25 µg/kg b.wt.) followed by intravenous infusion of 4—15 µg digoxin/kg/hr. in saline at a constant rate during the experiment. Blood and urine samples were drawn 30 min. after the priming dose and subsequently at 30 min. intervals (*Atef & Rasmussen* 1975).

The binding of digoxin to plasma proteins was estimated in vitro by adding digoxin to samples of plasma and in vivo i.e. in plasma samples from the clearance experiments. The protein binding was determined by ultrafiltration (*Atef & Rasmussen*).

Digoxin in plasma, urine and in ultrafiltrates of plasma was estimated by radioimmunoassay (*Steiness* 1974), which also includes metabolites of digoxin.

The endogenous creatinine clearance was used to express the glomerular filtration rate in swine (*Gyrd-Hansen*) as well as in goats (*Jørgensen & Rasmussen* 1972, *Atef & Rasmussen*). Clearance of creatinine in swine is equal to clearance of inulin (*Gyrd-Hansen*), while clearance of creatinine in goats is a little higher than clearance of inulin (*Atef & Rasmussen*). Creatinine was estimated colorimetrically (*Bonsnes & Taussky* 1945), and urea was estimated by the microdiffusion method (*Conway* 1950).

The pH of the blood and urine was measured immediately after each experiment by means of a potentiometer with glass electrode (Radiometer, Copenhagen) at 37°C.

The statistical calculations were done in accordance with standard methods (*Kemp* 1955), and the results are given as the

means ± s.e.m. The relationships between the renal clearance of digoxin and the concentration of non-protein-bound digoxin in plasma, urine pH and urine flow rate were examined by means of correlation matrices and multiple regression analyses (*Dixon* 1967).

RESULTS

Protein-binding

Table 1 shows the in vitro and in vivo binding of digoxin to plasma proteins in swine and goats. The binding of digoxin after addition to plasma (in vitro) was consistently lower than in plasma from animals administered digoxin (in vivo) ($P < 0.01$). The plasma protein-binding was both in vitro and in vivo independent of the plasma digoxin concentrations (1—10 ng/ml).

Table 1. Binding of digoxin to plasma proteins.

	Plasma from swine		Plasma from goats	
	in vitro	in vivo	in vitro	in vivo
Number of estimations (n)	6	9	15	9
Concentration of digoxin ng/ml, range	2.5—5.0	1.1—8.7	2.0—8.0	1.0—9.8
% bound (mean	29	43	34	50
± s.e.m.)	4	2	4	4

Renal clearance

The results of the simultaneous estimations of the clearances of endogenous creatinine, urea, digoxin and non-protein-bound digoxin in plasma and other experimental data are shown in Tables 2 and 3.

From Tables 2 and 3 it is seen that the clearance of endogeneous creatinine is the same in swine and goats. Further the tables show that the clearance of urea is around 50 % of the creatinine clearance in both species.

Digoxin in swine

From Table 2 it is seen that the plasma clearances of digoxin and that of the non-protein-bound digoxin ($Clear_{Dig.ultr.}$) were

Table 2. Clearance of endogenous creatinine, urea and digoxin in swine.

Experiment and swine no.	Body weight kg	Urine flow rate ml/min./10 kg b.wt.	Urine pH mean (range)	Plasma digoxin ng/ml	Clearance ml/min./10 kg b.wt.				Clearance ratio		
					creatinine	urea	digoxin	dig.ultr.	urea / creatinine	digoxin / creatinine	dig.ultr. / creatinine
1-F1	80	0.20	5.49 (5.40—5.60)	1.9	25	13	4	8	0.54	0.18	0.31
2-F1	80	0.12	6.84 (6.60—7.40)	7.2	28	11	4	7	0.39	0.13	0.24
3-F5	43	0.17	5.75 (5.41—5.85)	2.6	22	10	6	10	0.44	0.26	0.47
4-F4	50	0.11	6.49 (6.00—7.05)	1.9	24	13	4	7	0.52	0.18	0.31
5-F6	52	0.13	5.66 (5.51—5.81)	6.1	19	8	3	5	0.41	0.15	0.26
6-F7	54	0.11	5.35 (5.17—5.60)	2.0	21	9	5	8	0.45	0.23	0.40
7-F8	46	0.07	5.94 (5.38—7.15)	1.4	22	9	3	4	0.42	0.11	0.19
8-F8	55	0.14	5.85 (5.50—6.15)	5.6	19	11	4	6	0.58	0.19	0.33
Mean					22	11	4	7	0.48	0.18	0.32
± s.e.m.					0.5	0.4	0.2	0.4	0.01	0.01	0.02

Table 3. Clearance of endogenous creatinine, urea and digoxin in goats.

Experiment and goat no.	Body weight kg	Urine flow rate ml/min./10 kg b.wt.	Urine pH mean (range)	Plasma digoxin ng/ml	Clearance ml/min./10 kg b.wt.				Clearance ratio		
					creatinine	urea	digoxin	dig.ultr.	urea / creatinine	digoxin / creatinine	dig.ultr. / creatinine
1-50	36	0.94	7.91 (7.73—8.13)	3.0	30	14	14	27	0.48	0.29	0.87
2-43	43	0.17	8.43 (8.35—8.50)	2.3	28	10	14	28	0.33	0.51	1.00
3-49	35	0.13	8.35 (8.30—8.40)	3.2	29	12	13	26	0.41	0.45	0.89
4-40	56	0.12	7.39 (6.80—7.90)	9.1	14	6	4	8	0.40	0.27	0.57
5-63	28	0.18	6.50 (5.60—7.19)	6.6	31	10	9	18	0.31	0.29	0.78
6-50	38	0.45	7.78 (6.94—8.28)	2.4	27	13	12	25	0.47	0.45	0.91
7-43	36	0.13	8.56 (8.45—8.60)	1.2	23	8	17	34	0.37	0.75	1.51
8-49	35	0.27	8.40 (8.01—8.54)	3.9	21	7	10	21	0.34	0.50	1.00
9-63	28	1.30	7.75 (7.04—8.89)	4.4	26	14	11	22	0.46	0.43	0.82
10-49	34	1.79	7.56 (7.36—7.65)	2.0	23	10	12	24	0.46	0.46	1.05
Mean					25	10	12	23	0.40	0.46	0.92
± s.e.m.					1	1	1	1	0.01	0.02	0.05

much lower than the clearance of endogenous creatinine (ratio column 11 and 12) and even lower than clearance of urea.

By means of multiple regression analysis a regression equation has been calculated. This equation gives the influence of the concentration of non-protein-bound digoxin ($C_{Dig.ultr.}$), the urine pH and the urine flow rate (V) on the renal clearance of digoxin in comparison to the clearance of endogenous creatinine. The regression equation was

$$\text{Clearance ratio} = \frac{\text{Clear}_{Dig.ultr.}}{\text{Clear}_{Cr.}} =$$

$$0.15 - 0.014\ C_{Dig.ultr.} + 0.009\ pH + 11.25\ V \quad \text{(eq. 1)}$$

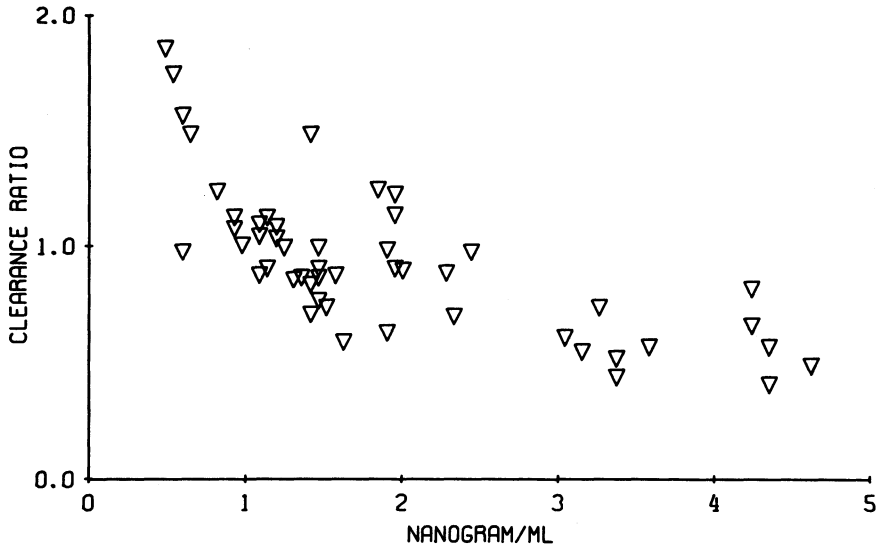
Correlation analysis showed significant correlation between the clearance ratio and the small variations in urine flow rate (V). The clearance ratio was neither significantly correlated to the concentration of non-protein-bound digoxin in plasma nor to the pH of the urine (Table 4). The variation of these three factors combined in the above mentioned regression equation could only explain 26 % of the variations of the clearance ratio.

Table 4. Correlation coefficients (r) between variables i.e. clearance ratio and the concentration of non-protein-bound drug in blood plasma ($C_{Dig.ultr.}$), the pH of urine and urine flow rate (V) in swine and goats.

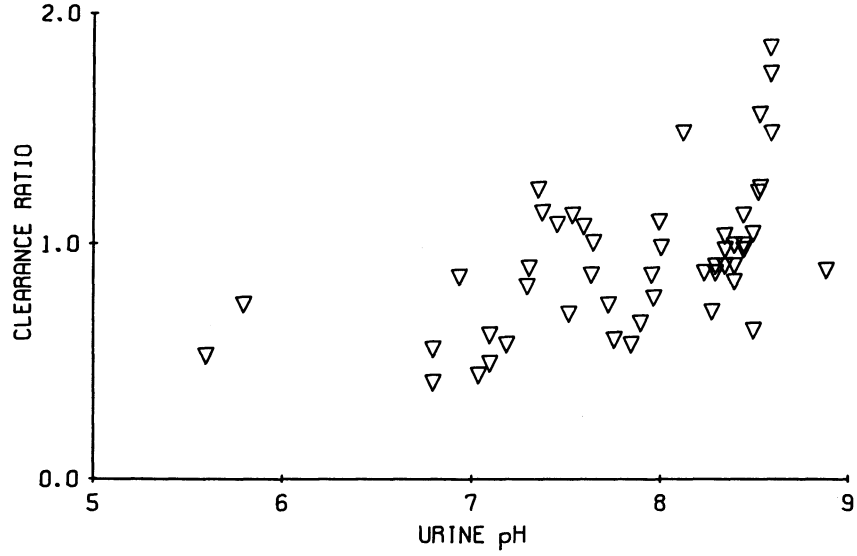
Species	Variables		r	P
Swine	$\frac{\text{Clear}_{Dig.ultr.}}{\text{Clear}_{Cr.}}$	$C_{Dig.ultr.}$	0.15	n.s.
		pH	0.15	n.s.
		V	0.48	< 0.001
Goats	$\frac{\text{Clear}_{Dig.ultr.}}{\text{Clear}_{Cr.}}$	$C_{Dig.ultr.}$	0.71	< 0.001
		pH	0.54	< 0.001
		V	0.02	n.s.

Digoxin in goats

Table 3 shows that the plasma renal clearance of digoxin was consistently lower than clearance of creatinine (column 11), while the clearance of non-protein-bound digoxin ($\text{Clear}_{Dig.ultr.}$) was



F i g u r e 1. Ratio between the clearance of non-protein-bound digoxin and endogenous creatinine in relation to the concentration of ultrafiltrable digoxin in plasma in goats.



F i g u r e 2. Ratio between the clearance of non-protein-bound digoxin and endogenous creatinine in relation to pH of urine in goats.

found lower, equal to or higher than that of creatinine (column 12).

By means of multiple regression analysis a regression equation has been calculated. The equation gave the influence of the concentration of non-protein-bound digoxin ($C_{Dig.ultr.}$), the urine pH and the urine flow rate (V) on the renal clearance of digoxin in comparison to the clearance of creatinine. The regression equation was

$$\text{Clearance ratio} = \frac{\text{Clear}_{Dig.ultr.}}{\text{Clear}_{Cr.}} =$$

$$0.78 - 0.182\ C_{Dig.ultr.} + 0.067\ pH - 0.638\ V \qquad \text{(eq. 2)}$$

Correlation analysis showed significant correlation between the clearance ratio and both the concentration of non-protein-bound digoxin ($C_{Dig.ultr.}$) and the pH of the urine, while the clearance ratio and the urine flow rate (V) were not significantly correlated (Table 4). The regression equation could explain about 54 % of the variations of the clearance ratio. The influence of the concentration of non-protein-bound digoxin in plasma and the urine pH on the renal clearance of digoxin is further demonstrated in Figs. 1 and 2. Fig. 1 shows that the renal excretion of digoxin in relation to clearance of endogenous creatinine diminishes when the concentration of non-protein-bound digoxin in plasma increases up to about 2 ng/ml, while further increase in concentration seems not to influence the clearance ratio. Fig. 2 shows that the excretion of digoxin increases with increasing pH of the urine.

DISCUSSION

Protein-binding

In a comparative study on 13 different species (*Baggot & Davis* 1973) it was found that in vitro binding of added digoxin to plasma proteins varied considerably; it was lowest (17 %) in rats and highest (40 %) in rabbits. In plasma from swine and goats 31 and 23 % of added digoxin was bound to plasma proteins, respectively. The in vitro binding found in the present study for swine was 29 ± 4 % (Table 1) and thus in good agreement with the findings by *Baggot & Davis* using an equilibrium

dialysing technique. However, the in vitro binding of digoxin to proteins in plasma from goats (34 ± 4 %) was higher than the results (23 ± 2 %) given by *Baggot & Davis*. The in vitro estimated binding of digoxin to proteins in plasma was significantly lower than the binding found in plasma samples obtained from animals treated with digoxin ($P < 0.01$, Table 1). A possible explanation for this difference between in vitro and in vivo estimations might be the presence in plasma from treated animals of one or more metabolites of digoxin which has a higher degree of protein binding than digoxin and was estimated as digoxin by the radioimmunoassay (*Steiness* 1974).

Renal clearance

The results concerning the creatinine clearance in Tables 2 and 3 showed that the average values are the same in swine and goats and are in agreement with earlier findings (vide *Gyrd-Hansen* 1968, *Atef & Rasmussen* 1975). From Tables 2 and 3 it is further seen that the clearance of urea varied between 31 and 58 % of creatinine clearance suggesting a considerable reabsorption of urea. These observations are also in accordance with earlier findings (*Gyrd-Hansen, Atef & Rasmussen*).

In evaluating the renal handling of digoxin three main mechanisms should be considered: glomerular filtration, active tubular secretion and back diffusion. The protein-bound fraction of digoxin cannot filter through the glomeruli of the kidneys, and therefore the renal excretory mechanism of digoxin must be evaluated on the basis of non-protein-bound digoxin in plasma.

From Table 2 it is seen that in pigs the clearance of non-protein-bound digoxin in plasma is always lower than clearance of endogenous creatinine (19—47 %) while in goats (Table 3) the clearance of non-protein-bound digoxin varied from 57 to 151 % of endogenous creatinine clearance. These variations in the clearance of digoxin and the quantitative differences between swine and goats have been analysed for influence of the concentration of digoxin in plasma, urine pH and urine flow rate. From the regression equation (eq. 1) it is seen that the renal clearance of digoxin in swine is not significantly influenced by the concentration of non-protein-bound digoxin in plasma and the pH of the urine, while there is a significant positive correlation between the clearance ratio and the urine flow rate (Table 4),

however, the variations in urine flow rate have been rather small. This indicates that glomerular filtration and back diffusion are involved in the renal handling of digoxin in swine. In contrast, the regression equation (eq. 2) shows that the renal clearance of digoxin in goats is significantly influenced by the concentration of non-protein-bound digoxin in plasma (Fig. 1) as well as by the urine pH (Fig. 2). On the other hand, the urine flow rate does not influence the clearance (Table 4). The remarkable decrease in digoxin renal clearance when the concentration in plasma increases (Fig. 1) shows that the excretory mechanism can be saturated indicating active tubular secretion. Thus, in goats the renal handling of digoxin involves in addition to glomerular filtration and back-diffusion also active tubular secretion. The active tubular secretion of digoxin found in goats is in agreement with recent findings in man (*Steiness*), while such an active secretion of digoxin has not been demonstrated earlier (vide *Steiness*).

The previous inability to demonstrate active tubular secretion of digoxin can partly be explained by the fact that approx. 25 % is bound to plasma proteins in man (*Lukas & DeMartino* 1969, *Ohnhaus et al.* 1972, *Baggot & Davis, Dengler et al.* 1973) and consequently not available for glomerular filtration.

As digoxin is a neutral compound it was expected that the renal handling and especially the back-diffusion of the drug should be independent of the pH in the urine. The results indicated that this was true in swine with acid urine (pH 5.4—7.2). Similar results were seen in goats with urinary pH of 5.6—7.2, while the renal excretion of digoxin increased, when the urinary pH was above 7.2 (Fig. 2). This indicated that digoxin behaved in vivo as a weak acid or that weakly acidic metabolites of the drug estimated as digoxin were formed. Experiments concerning mammary excretion of digoxin in goats showed that the concentration of non-protein-bound digoxin in milk was a little lower that non-protein-bound digoxin in plasma (*Rasmussen et al.* 1975). According to the theory of non-ionic-diffusion through the mammary gland epithelium (*Rasmussen* 1966, 1971) the mentioned results further emphasize the assumption that digoxin in vivo behaves as a weak acid or that weakly acidic metabolites of digoxin are formed.

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REFERENCES

Atef, M. & Folke Rasmussen: Mammary and renal excretion of sulphadiazine in goats. Zbl. Vet.-Med. A. 1975, *22*, 500—509.

Baggot, J. D. & L. E. Davis: Plasma protein binding of digitoxin and digoxin in several mammalian species. Res. Vet. Sci. 1973, *15*, 81—87.

Bertler, A. A. & A. Redfors: Plasma glycoside level in relation to digoxin toxicity. Proc. 5th Int. Pharmacol. Meet., San Francisco 1972.

Bloom, P. M. & W. B. Nelp: Relationship of the excretion of tritiated digoxin to renal function. Amer. J. med. Sci. 1966, *251*, 133—144.

Bonsnes, R. W. & H. H. Taussky: On the colorimetric determination of creatinine by the Jaffe reaction. J. biol. Chem. 1945, *158*, 581—591.

Conway, E. J.: Microdiffusion Analysis and Volumetric Error. 3rd Ed., Crosby, Lockwood & Son Ltd., London 1950, p. 152.

Dengler, H. J., G. Bodem & K. Wirth: Pharmakokinetische Untersuchungen mit H^3-Digoxin und H^3-Lanatosid beim Menschen. (Pharmacokinetic studies on H^3-digoxin and H^3-lanatoside in man). Arzneimittel-Forsch. (Drug Res.) 1973, *23*, 64—74.

Dixon, W. J.: Biomedical Computer Programs. 2nd Ed. Univ. California Press, Berkeley & Los Angeles 1967, p. 258.

Doherty, J. E., W. J. Flanigan & W. H. Perkins: Tritiated digoxin excretion in human subjects following renal transplantation. Circulation 1968, *37*, 865—869.

Doherty, J. E., W. J. Flanigan, R. M. Patterson & G. V. Dalrymple: The excretion of tritiated digoxin in normal human volunteers before and after unilateral nephrectomy. Circulation 1969, *40*, 555—561.

Ewy, G. A., G. G. Kapadia, L. Yao, M. Lullin & F. I. Marcus: Digoxin metabolism in the elderly. Circulation 1969, *39*, 449—453.

Gyrd-Hansen, N.: Renal clearances in pigs. Acta vet. scand. 1968, *9*, 183—198.

Jørgensen, S. Tue & Folke Rasmussen: Renal ekskretion af sulfanilamid, sulfadimidin, sulfadoxin og sulfamethoxazol hos geder. (Renal excretion of sulphanilamide, sulphadimidine, sulphadoxine and sulphamethoxazole in goats). Nord. Vet.-Med. 1972, *24*, 601—611.

Kemp, T.: Statistik for medicinere. (Statistics for physicians). Munksgaard, København 1955.

Lukas, D. S. & A. G. DeMartino: Binding of digoxin and related cardenolides to human plasma proteins. J. clin. Invest. 1969, *48*, 1041—1053.

Ohnhaus, E. E., P. Spring & L. Dettli: Protein binding of digoxin in human serum. Europ. J. clin. Pharmacol. 1972, *5*, 34—36.

Rasmussen, Folke: Studies on the mammary excretion and absorption of drugs. Thesis. C. Fr. Mortensen. Copenhagen 1966.

Rasmussen, Folke: Excretion of drugs by milk. *In* Brodie, B. B. & J. Gillette: Handbook of Experimental Pharmacology. Springer Verlag 1971, 28: 1, 390—402.

Rasmussen, Folke, M. Nawaz & E. Steiness: Mammary excretion of digoxin in goats. Acta pharmacol. (Kbh.) 1975, *36*, 377—381.

Steiness, E.: Renal tubular secretion of digoxin. Circulation 1974, *50*, 103—107.

Steiness, E., O. Svendsen & Folke Rasmussen: Plasma digoxin after parenteral administration. Clin. Pharmacol. Ther. 1974, *16*, 430—434.

SAMMENDRAG

Renal ekskretion af digoxin hos svin og geder.

Den renale ekskretion af digoxin er blevet undersøgt hos svin og geder. Undersøgelsen viste, at den renale ekskretion af ikke-proteinbundet digoxin hos svin var lavere end filtrations clearance bestemt ved hjælp af kreatinin. En korrelationsanalyse (tabel 4) viste, at den renale clearance af digoxin hos svin i disse forsøg var uafhængig af koncentrationen af ikke-protein-bundet digoxin i plasma og af urinens pH, mens der var en tydelig positiv korrelation mellem clearance og diurese (tabel 4). Forsøgene på geder viste, at den renale clearance af digoxin var signifikant afhængig af koncentrationen af ikke-proteinbundet digoxin i plasma og af urinens pH (tabel 4). På grundlag af de opnåede resultater konkluderes, at såvel glomerular filtration som tilbagediffusion er involveret i den renale ekskretion af digoxin hos både svin og geder. Hos geder er der tillige påvist aktiv tubulær sekretion af digoxin.

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